



Nano-structured Metal-Enhanced Fluorescence (MEF-spot)-Based Sensing, Imaging and Assay

Summary

This invention describes an improved method for conducting a bioassay using fluorescence intensity or lifetime fluorescent emissions. This new method avoids the need of complex enzymatic and biochemical amplification of the fluorescence signal. Although it has applicability over a wide range of bioassays, the method has particular application in sensing, imaging and assaying cytokines. MEF-spot allows for the rapid detection of cytokines and other proteins secreted by a living cell in small quantities, in the presence of living cells and without directly rinsing the sample before conducting the bioassay. In addition to imaging individual cell secretions, this approach allows the visualization of cell-cell interactions in co-cultured cells.

Key Investigator

Henryk Szmazinski
 Joseph R. Lakowicz

Field

Fluorescence Imaging
 Research Tool

Technology

Cytokine assay
 Immunoassay

Advantages

Does not require enzymatic or biochemical amplification

Assay performed in the presence of living cells and other analytes

Cytokine detection of ~ 0.01 ppb

Assay cell-cell interactions in real time

Status

Available for licensing and commercial development

Patent Status

US 14/359,343
 US 14/965,560
 PCT/US2012/066451

UMB Docket Reference

HS-2012-047; HS-2015-098

External Reference

Szmazinski et al. (2010) *J Phys Chem C Nanomater Interfaces*. 114(16):7236-41.

Szmazinski et al. (2010) *J Phys Chem C Nanomater Interfaces*. 114(49):21142-49.

Immunoassay Market - Global Forecast to 2021. November 2016. Report Code MD4751.

Market

Current techniques for secreted cytokine assays include ELISA, flow cytometry, RT-PCR, ELISPOT, and FLUOROSPOT. End users for these bioassays include research labs, hospitals, blood banks, CROs, biotech companies, and clinical labs. MEFspot has the potential to be used as a life science reagent, in clinical diagnostics and therapeutics, and in drug development. In 2016, the global immunoassay market reached \$16.16 billion at a compound annual growth rate of 8.2%. It is expected to reach \$25.45 billion by 2021.

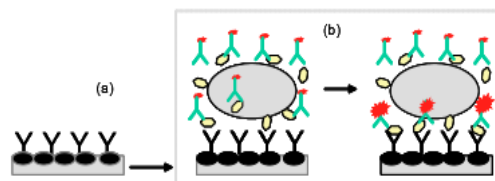
Technology

The sensing and imaging principles of MEFspot rely on the enhancement of fluorescence intensity or changes in fluorescence decay of an analyte bound to a surface with a fluorophore-labeled detection probe. The sensing bioactive surface comprises the capture biomolecule attached to the surface with plasmonic nanostructures. This technology also includes the fabrication procedure of plasmonic substrates. The binding of the detection probe to the bioactive surface with an analyte increases fluorescence intensity up to 200x and a decrease in fluorescence lifetime by more than 5x. The enhancement of fluorescence intensity allows for direct sensing and imaging of biomolecule interactions.

MEFspot is a significant advance in single cell assays as its sensitivity is comparable to enzyme-linked immunospot, but it uses a substantially simpler procedure that still allows for real-time monitoring and quantitative analysis.

Technology Status

The technology has been extensively researched by the inventors. Peer review articles demonstrating the technique on numerous analytes have been published.



Scheme of MEFspot procedure.

(a) Immobilization of capture antibody specific for secreted cytokine on the MEF substrate. (b) Incubation with cells stimulated for cytokine secretion in the presence of dye-labeled detection antibodies. Fluorescence in a sandwich complex on the surface is amplified and distinct from that in solution (unbound to the surface).